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# The olive goblet: *Peziza oliviae,* a new cup fungus growing underwater in Oregon

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Abstract — A new species, *Peziza oliviae*, is described from stream habitats in the Oregon Cascade Mountains (USA) based on morphology, habitat, and ITS and LSU nrDNA sequence data. This new member of the *Ascomycota* grows underwater on sticks and decomposing wood as well as near running water where the woody substrate is saturated.

Key words — *Pezizaceae,* underwater, monitoring, survey, manage

## Introduction

Surveys for rare and endangered fungi in Oregon continue to be part of management practices, which now include stream habitats in search protocols for rare macrofungi that fruit underwater, such as *Vibrissea truncorum* (*Ascomycota*) and *Psathyrella aquatica* (*Basidiomycota*) (Frank et al. 2010; Oregon Biodiversity 2010). While only one gilled mushroom, *P. aquatica*, from the Rogue River in southern Oregon, is known to grow and fruit underwater, several ascomycetes fruit when submerged. *Cudoniella clavus* forms tan to gray fruiting bodies on submerged sticks, while *Vibrissea truncorum* and *Mitrula* spp. form orange caps on submerged sticks as well as on wet woody debris near water. Sessile macrofungi (e.g., *Vibrissea filisporia*, *Scutellinia scutellata, Pachyella clypeata*, *Adelphella babingtonii*) fruit underwater in streams in Oregon, and the new genus *Aquapeziza* (*Pezizaceae*) was recently described for a cup fungus fruiting underwater in China (Pfister 1973; Wang et al. 2005; Pfister et al. 2008; Hu et al. 2012). Aquatic microfungi are also classified in the *Oomycota*, *Chytridiomycota*, and aquatic hyphomycetes as well as new genera in the *Leotiomycetes* and *Sordariomycetes* from Florida and Costa Rica (Raja et al. 2009; Ferrer et al. 2012). Documentation of new and unusual fungal biodiversity provides target descriptions and DNA barcodes useful for monitoring and conservation (Halme et al. 2012).

In the broad sense, *Peziza* historically encompassed a variety of ascomal morphologies, including the archetypal sessile cup, stalked goblets, and convoluted truffle-like sequestrate fungi. Molecular data indicate that *Peziza* s.l. is polyphyletic and should be separated into several distinct genera, with the core group (*Peziza* s.s.) surrounding the generic type, *P. vesiculosa*. Fungi in the core group are saprotrophic and characterized by an amyloid ring at the tips of the asci, which form a palisade with thinner paraphyses along a more or less cup-shaped hymenium. They are represented by ellipsoid spores that may be smooth or ornamented; a stalk may be present but is usually not prominent, forming just a point of attachment to the substrate (Dennis 1968; Korf 1973; Breitenbach & Kränzlin 1984; Tylutki 1979; Hansen et al. 2001, 2002).

During surveys for rare species in central Oregon, I repeatedly collected an olive-brown stalked cup fungus growing underwater in small streams in the Willamette National Forest. Previously, no species of *Peziza* s.s. have been reported growing underwater in Oregon. Aquatic fruiting is such an unusual phenomenon that mycologists apply special scrutiny to confirm that a fungus has not originally fruited above water and subsequently fallen in or become submerged in rising waters. The fungus, which does not match any previously described species on survey lists or in scientific literature, is described here as *Peziza oliviae*.

## Materials & methods

Streams, riverbanks, and saturated woody substrates were surveyed for macrofungi during May–November 2011 and May–August 2012 in the Willamette and Mount Hood National Forests. Site locations (UTM NAD83 Zone 10) were determined and recorded using a Garmin 450 portable GPS unit. Collections were mapped, described in the field. Photographs of fresh material were taken with a Canon T2i digital camera (field) or under a Leica MZ75 dissecting microscope (laboratory). Fresh hand sections mounted in distilled water and Melzer's reagent were viewed under a Leica DMLB compound microscope. Thirty spores from each of eight collections were measured at 1000×. All microscopic measurements were made from sections mounted in distilled water.

DNA was extracted, amplified, and sequenced following Frank et al. (2010): fresh fungal tissue was ground with micropestles in CTAB buffer, extracted with chloroform, and amplified with PCR primers ITS1f, ITS4, and TW13. Internal primers ITS1 and ITS4r were used for sequencing reactions. After sequences were edited with Chromas 1.45 (McCarthy 1998), contiguous sequences were assembled in Sequencher v4.7 (Gene Codes Corp. Ann Arbor, MI) and compared to other fungal ITS and LSU sequences in GenBank with BLAST (Altschul et al. 1990). MAFFT and ClustalX were used to generate multiple sequence alignments (Katoh et al. 2002; Thompson et al. 1997). Alignments were edited manually using BioEdit and Mesquite (Hall 1999; Maddison & Maddison 2011). Phylogenetic trees, using maximum likelihood with 1000 bootstrap replicates, were generated using fastDNAml (Felsenstein 1981; Olsen et al. 1994) through the Mobyle portal of the Pasteur Institute (http:/mobyle.pasteur.fr). ML analyses using

settings that correspond to the HKY85 model, and parsimony analyses with 1000 bootstrap replicates and 1000 jack-knife replicates, were generated using PAUP* 4.10b10 (Swofford 2002). Consensus trees with 50% majority-rule were generated using the tree-bisection-reconnection branch-swapping algorithm. All characters were given equal weight; gaps were treated as missing.

Of the 41 aligned ITS sequences, five were generated from collections of *Peziza oliviae*, one was generated from *Peziza* sp. collected in southern Oregon, and 35 were obtained from GenBank. ITS and LSU rDNA sequences have been deposited in GenBank; ITS alignment and trees were submitted to TreeBase.org.

## Results

Ten collections of *P. oliviae* were made during July–October 2011 and June–August 2012. Ascocarps were found growing from stream bottoms submerged 10–25 cm in running water or on saturated wood near running water in first order streams in the Santiam and Breitenbush watersheds at 900–1400 m elevations.

ITS nrDNA was amplified and sequenced for five collections of *P. oliviae* and for one morphologically similar *Peziza* sp. collected underwater in southern Oregon (JLF2167). LSU nrDNA was amplified and sequenced for three collections of *P. oliviae* and for *Peziza* sp. (JLF2167). Sequence data have been deposited in GenBank (JX415339–JX415342 and KC916727–KC916729).

Edited alignments of 41 ITS sequences generated consensus trees using parsimony with 774 total, 256 constant, 117 variable, and 401 parsimony-informative characters. Maximum Likelihood and Maximum Parsimony analyses support the molecular uniqueness of this new species, with *P. oliviae* nearest to the European *P. lohjaoensis* (Plate 1). Maximum Parsimony analysis and pairwise comparisons of LSU sequence data confirm the placement of *P. oliviae* in *Peziza* s.s. Alignments and trees are available at http://purl.org/phylo/treebase/phylows/study/TB2:S14001.

Morphological and molecular analyses support the designation of this unusual stalked cup as a new species. This cup fungus grows underwater on sticks and wood embedded in the sediments, pebbles, and cobble of mountain stream bottoms and above water on saturated wood near cold mountain streams in the Oregon Cascades. It has a prominent stalk, olive-brown color, operculate asci with amyloid tips, smooth nonguttulate ellipsoid spores, and ITS and LSU nrDNA sequences that place it in *Peziza* s.s. (Plate 1).

## Taxonomy

***Peziza oliviae*** J.L. Frank, **sp. nov.** Pl. 2

MycoBank MB803430

Differs from the genetically related *Peziza lohjaoensis* by its smooth spores and North American distribution and from other *Peziza* spp. by its submerged habitat.

Plate 1. Maximum Likelihood tree using ITS nrDNA showing the placement of *Peziza oliviae* in *Peziza* s.s. 23,336 rearrangements tried, score of best tree = 9722.72452. MP Bootstrap numbers greater than 50% are included above branches; analyses performed in PAUP* using 1000 bootstrap replicates.

Type: United States, Oregon, Marion Co., Willamette NF, Dunlap Creek, UTM E571615 N4962062, elevation 990 m, underwater in stream, 15 Jul 2011, JLF2091 (**Holotype**-OSC148300; GenBank JX415340).

Etymology: for Doris Olivia for help finding this cryptic cup fungus; also reflecting the olive hue of the ascomata.

Ascomata olive to golden-brown stalked cups 7–25 mm tall, 8–40 mm diam, margin crenulate. Stipe 1–10 × 3–7 mm, external surface roughened, glabrous. Ascomal morphology varies from stalked cup or goblet-shape with upturned margin to a nearly sessile slightly convex disc with a short but prominent stalk.

Subhymenium 15–25 µm thick, composed of small isodiametric cells 4–10 µm. Medullary excipulum 500–1800 µm thick, composed of large inflated

isodiametric cells 45–110 × 35–85 µm. Ectal excipulum 50–150 µm thick, composed of elongate and contorted cells 20–40 × 15–35 µm with olive-brown pigmentation.

Asci 250–380 × 16–22 µm, cylindrical, operculate, arranged in a regular palisade with amyloid tips forming a distinct blue ring zone. Paraphyses 3–5 µm diam, with enlarged clavate tips 5–8 µm diam equal to or extending slightly (3–8 µm) beyond the asci, septate with septa 15–40 µm apart. Spores nonguttulate smooth ellipsoid 19.5–24 × 9.5–12 µm, 8 per ascus.

Ecology & distribution – small streams in the Cascade Range of north central Oregon between 800 and 1500 m elevation, associated with dead woody debris embedded in stream bottoms or on saturated wood, at the stream surface or on stream banks; June through October.

Additional specimens examined —**UNITED STATES. Oregon:** Marion Co., Willamette NF: underwater in Dunlap Creek, UTM E584752 N4964113, elev. 990 m, 15 Jul 2011, JLF2087 (OSC148297); UTM E571892 N4962471, elev. 1070 m, JLF2088 (OSC148298; GenBank JX415339); UTM E571641 N4962234, elev. 1010 m, JLF2089 (OSC148299); UTM E577193 N4964125, elev. 1050 m, 27 Jul 2011, JLF2118 (OSC148301; GenBank JX415341); UTM E584753 N4964113, elev. 1212 m, 10 Aug 2011, JLF2140 (OSC148302); UTM E559896 N4957372, elev. 1270 m, 15 Oct 2011, JLF2189 (OSC148303); UTM E571880 N4962471, elev. 1090 m, 27 Jun 2012, JLF2474 (OSC148304; GenBank KC916728); UTM E571935 N4962478, elev. 1120 m, 27 Jul 2012, JLF2541 (OSC148307); below Scorpion Mtn. underwater in E. fork of Humbug Creek, UTM E578141 N4963797, elev. 1295 m, 29 Jun 2012, JLF2479 (OSC148305; GenBank KC916729); underwater in upper Byars Creek, UTM E568438 N4959910, elev. 1240 m, 26 Jul 2012, JLF2538 (OSC148306).

Other species examined: ***Peziza* sp.**: **UNITED STATES. Oregon:** Jackson Co., Rogue River-Siskiyou NF, underwater in the Rogue River, UTM E540414 N4745695, elev. 995 m, 7 Sep 2011, JLF2167 (OSC148308; GenBank JX415342, KC916727)

Comments– *Peziza oliviae* is distinguished from other species in *Peziza* s.s. by the combination of a stipe, smooth nonguttulate spores, olive-brown pigmentation, its growth underwater or on saturated woody substrates, and its western United States distribution. Among similar species, *P. vesiculosa* lacks a prominent stalk and grows on dung, *P. domiciliana* forms smaller biguttulate spores, *P. echinospora* produces spiny spores, and *P. ammophila* grows in sand and has a cracked margin. Its closest relative, *P. lohjaoensis,* is separated by a 20bp difference (>3%) in the ITS1+ITS2 region, warty spores, growth on decaying hardwood litter, and a European distribution (Hansen et al. 2002). Another stalked *Peziza* sp. (JLF2167), collected underwater in the upper Rogue River in southern Oregon, appears to be a distinct species in *Peziza* s.s., but more collections are needed to confirm its taxonomic position and status (Plate 1).

*Peziza oliviae* differs from other aquatic or semiaquatic members of the *Pezizaceae* with amyloid asci as follows: *Aquapeziza* forms globose spores,

Plate 2. *Peziza oliviae.* A: ascocarp in situ underwater (JLF2189). B: ascocarp with irregular crenulate margin (JLF2189). C: ascocarp (JLF2091). D: ascocarp cross-section (JLF2140). E: spores in asci, amyloid in Melzer's toward the operculate tip (JLF2091). F: spores (JLF2474). Bars: A, B = 1 cm; C = 0.5 cm; D = 1 mm; E,F = 10 µm.

and *Pachyella* and *Adelphella* are non-stipitate and have gelatinous tissues. While the aquatic *Cudoniella clavus* is not closely related, it has many forms including a tan stalked cup that can macroscopically resemble *P. oliviae,* so that surveyors might mistake this for *P. oliviae* in the field. However, *C. clavus* is easily distinguished microscopically by its smaller spores and non-amyloid inoperculate asci.

*Peziza oliviae* appears to be rare and associated with undisturbed forest habitats. The streams in which *P. oliviae* was collected contained aquatic insect larvae and other ascomycetes including *Vibrissea truncorum*, *V. filisporia*, *C. clavus*, *Scutellinia scutellata,* and *Adelphella babingtonii*. These streams are small and do not experience regular disturbances, such as major sediment moving floods and scouring, as do larger stream systems. These fast-moving small streams, found at 800–1500 m in the central Oregon Cascade Mountains, maintain relatively consistent conditions with temperatures and moisture levels not fluctuating nearly as much as the surrounding terrain. Ascocarps were collected at the bottom of streams attached to woody substrates embedded in gravel and cobble. These fungi did not fruit terrestrially and thus were not subsequently inundated by rising waters. Some fruiting bodies were collected from saturated old logs and log fragments from large conifers (probably *Pseudotsuga menziesii* or *Tsuga heterophylla*) near the water. Growth on dead wood suggests that *P. oliviae,* like other species in *Peziza* s.s. (Hansen et al. 2002), is saprotrophic.

The discovery of this unusual species documents new biodiversity in healthy old-growth forests, underscores the importance of monitoring and maintaining such habitats, and adds to the list of species that represent measurable units affected by adaptive management practices. Its common name, "the olive goblet" (based on its chalice- or grail-like appearance), should help forest managers and surveyors find and identify this unique cup fungus.

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